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1. American Journal of Pathology:
   1993 Feb, 142(2):403-412
   1993, 143(4):1150-1158
   1984, 114(3):454-460
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Differentiation
Ontogeny and Neoplasia

Subtyping of epithelial cells of normal and metaplastic human uterine cervix, using polypeptide-specific cytokeratin antibodies

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Abstract. The aim of the present study was to explore the histogenesis of metaplastic cells in the human uterine cervix. In a previous study [20] we demonstrated that squamous cervical metaplasia expresses a unique set of cytokeratin polypeptides different from that expressed by the various normal epithelial elements of both the exo- and endocervix. It was thus proposed that the formation of squamous metaplasia represented a new route of differentiation. In the present study we further investigated this aspect by expanding the battery of monoclonal antibodies directed against specific cytokeratin epitopes used for immunohistochemical labelling. The antibodies used were: *KS-1A3*, which specifically stains cytokeratin polypeptide no. 13; antibody *KS-2.1*, which is an anti-cytokeratin reacting with pseudostratified transitional and some simple epithelia; and antibody *KS-B17.2* reacting with cytokeratin polypeptide no. 18. Examination of the staining patterns obtained with these antibodies revealed specific staining of ciliated cells with antibody *KS-2.1* and of endocervical reserve cells with antibody *KS-1A3*. In 6 out of 19 cases tested reserve cells were also stained with antibody *KS-2.1*. These results enabled us to distinguish between at least four types of cells residing within the simple epithelium of the endocervix, namely columnar nonciliated cells, ciliated cells, and two subpopulations of reserve cells. Since metaplasia was positively stained by antibodies *KS-1A3* and *KS-2.1*, we propose that the endocervical reserve cells that express cytokeratin polypeptide no. 13 are most probably the cells from which endocervical metaplasia is derived.

Introduction

Antibodies reactive with the subunits of intermediate filaments have been extensively used over the last several years for tumor diagnosis, helping to distinguish between poorly differentiated neoplasms of mesenchymal, epithelial, myogenic, neuronal or astrocytic origins [11, 16, 24, 28, 31, 35, 36]. More recently, with the introduction of specific antibodies reactive with subsets of cytokeratin polypeptides, it became possible to distinguish between epithelial tumors of different cellular origins as well as between those displaying distinct degrees of differentiation [3, 13, 22, 26, 30, 37, 40, 43]. Of particular interest in those studies was the observation that as tumors develop, changes may occur in the pattern of cytokeratin polypeptides they express [25].

A most useful system for the study of differentiation-dependent modulation of cytokeratin expression comprises metaplastic lesions displaying transformation of one differentiated cell phenotype into another [8, 9, 27, 41]. In previous studies we have particularly focused on metaplastic processes in the human uterine cervix [21] and salivary glands [19]. The simple epithelium of the endocervix at the transformation zone (the exocervical – endocervical junction) or throughout the endocervix undergoes stratification [8, 10]. Using a battery of cytokeratin-specific monoclonal antibodies we have shown that the labelling pattern of the metaplastic tissue was different from that expressed in any of the normal cellular constituents of the cervix [21]. These changes were consistent with the acquisition of a "stratified" route of differentiation, without completely losing the cytokeratins typical of simple epithelia. We have proposed that cervical squamous metaplasia represents a distinct route of differentiation in endocervical reserve cells, which normally apparently differentiate into the various types of columnar cell present in the endocervix. Several questions have, however, remained open, in particular those concerning the complete cytokeratin profile of the endocervical reserve cells, from which the metaplasia is believed to develop [8, 10, 26, 39]. Direct examination of the specific cytokeratin polypeptides expressed by metaplastic cells presents some major difficulties, since these cells cannot be readily isolated or microdissected for a direct biochemical analysis. Furthermore, immunohistochemical labelling may provide only partial answers, since the labelling pattern for specific cytokeratins in tissue sections may be affected not only by the presence or absence of a particular polypeptide but also by the availability of the particular epitope recognized by the antibodies used [14, 46].

To approach these problems we have, in the present study, extended the battery of cytokeratin-specific antibodies and thus obtained new information concerning the metaplastic process and on the diversity of the various cell types lining the normal and metaplastic endocervix.

Methods

Tissues. The human tissues studied here were obtained from routine operations performed at the Kaplan Hospital in Rehovot. The cervical tissues were obtained from 20 patients and the other tissues from at least 2 patients each.

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The samples were snap-frozen in isopentane precooled in liquid nitrogen and then stored at −70° C until being used, as previously described [1, 12].

Antibodies. The three new monoclonal antibodies described in this paper were prepared by fusion of myeloma cells (NS0/1) with spleen cells taken from mice that had been injected with cytoskeletal preparation of either A-431 cells (antibodies *KS-1A3* and *KS-2.1*) or BMGE cells (antibody *KS-B17.2*). The fusion was carried out as described previously [17, 20] and positive hybridoma cultures were selected by immunocytochemical labelling of cultured A-431 cells (originating in human epidermoid carcinoma of the vulva). The new monoclonal antibodies used in the present study were:

1. *KS-1A3* antibody, reacting with human cytokeratin polypeptide no. 13 (numbers are according to the classification of Moll et al. [29]).

2. *KS-2.1* antibody, a cytokeratin antibody, based on its immunocytochemical reactivity with the cytokeratin network in cells and tissues. The fine polypeptide specificity of this antibody is not defined, since it does not react with electrophoretically separated polypeptides in Western-blot analysis.

3. *KS-B17.2* antibody, an antibody selectively reactive with human cytokeratin polypeptide no. 18.

The different monoclonal antibodies used were usually applied for immunohistochemical labelling as undiluted hybridoma culture supernatants. The secondary antibodies were affintiy-purified goat antibodies raised against mouse Fab'2, and conjugated to lissamine rhodamine sulfonyl chloride (or dichlorotriazinyl amino fluorescein in double staining) as previously described [2, 18]. In addition, we have used a monoclonal antibody directed against human tubulin to identify ciliated cells (kindly provided by Dr T. Kreis, Heidelberg, Federal Republic of Germany) [23], and rabbit antibodies directed against cytokeratin and vimentin.

Immunohistochemistry. Frozen sections of 5 μ were acetone-fixed and immunolabelled as previously described [1, 12]. Immunofluorescently labelled sections were examined using a Zeiss Axiophot microscope equipped for epifluorescence, using a plan-apochromat × 40/1.0 oil iris objective. Parallel sections, as well as paraffin sections of the specimens, were stained with hematoxylin-eosin.

Gel electrophoresis and immunoblotting. Cell cultures of A-431 and PLC (human hepatoma cell line containing cytokeratin polypeptides 8 and 18 and some vimentin) were extracted with high-salt buffer [1], and analysed by two-dimensional gel electrophoresis [34] followed by immunoblotting [44].

Immunoelectronmicroscopy. Frozen sections (20 μm thick) were cut at −20° C in a Frigocut 2700 Cryostat (Jung-Reichert, FRG), placed on clean glass coverslips, air-dried and acetone-fixed. The dry sections were subsequently labelled with immunoperoxidase [42], postfixed with 2% glutaraldehyde and 1% OsO_4, dehydrated and embedded in epon blocks. After 3 days polymerization at 60° C, appropriate areas and orientations were selected, and sections were cut using a Reichert MC4 ultramicrotome with a diamond

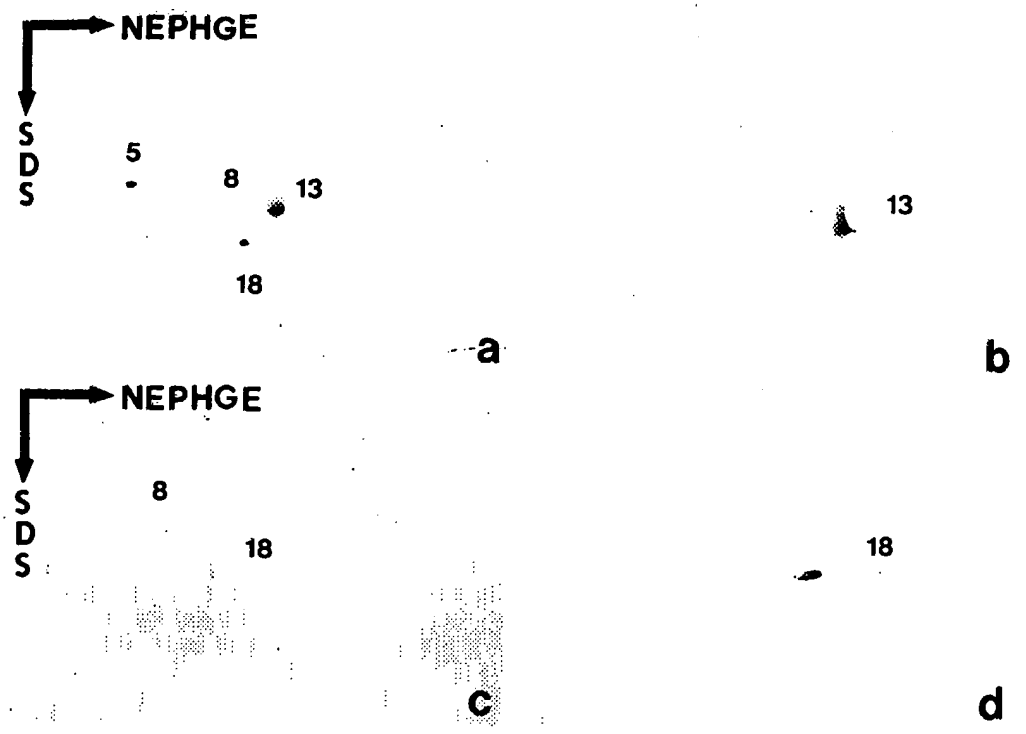


Fig. 1a–d. Two-dimensional gel electrophoresis of cytoskeletal polypeptides transferred to nitrocellulose paper. **a** Cytoskeletal polypeptides of A-431 cells stained with Ponceau-red. **b** Autoradiography of the same blot as in **a**, after reaction with the monoclonal antibody *KS-1A3* and ^{125}I-labelled goat antibodies to mouse Ig. **c** Cytokeratins of PLC cells stained with Ponceau-red. **d** Autoradiograph of the same blot as in **c**, after reaction with the monoclonal antibody *KS-B17.2*. Note that antibody *KS-1A3* reacted with cytokeratin no. *13* and *KS-B17.2* reacted with cytokeratin no. *18*

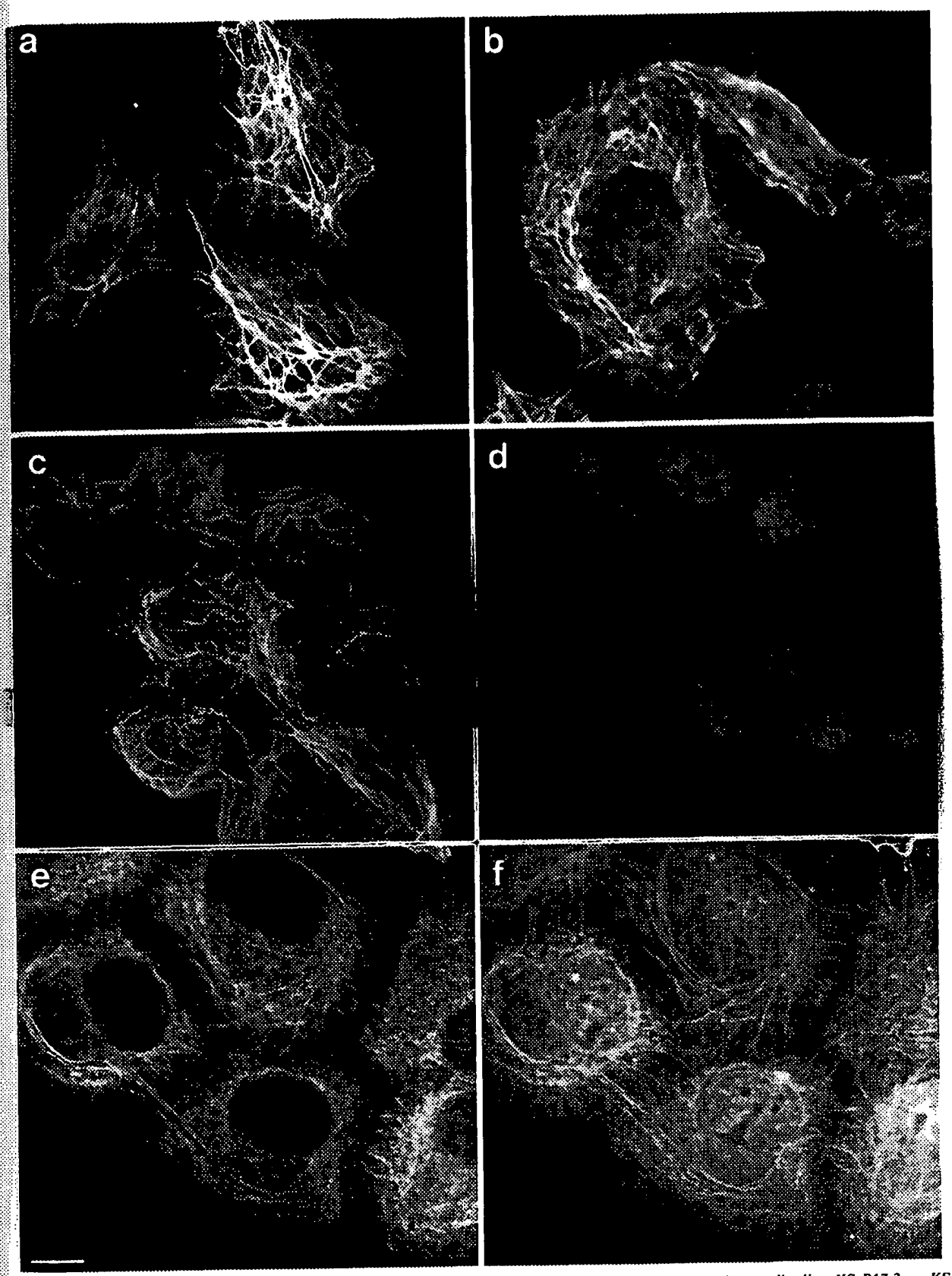


Fig. 2a–f. Immunofluorescence staining of human epidermoid carcinoma cell line A-431 cells using antibodies *KS-B17.2* **a**, *KS1A3* **b**, double labeling of the same cells with antibodies *KS-2.1* (**c**) and Rabbit anti-vimentin (**d**), double labeling of the same cells with antibodies *KS-2.1* (**e**) and rabbit anti-cytokeratin polypeptides (**f**). Note the positive staining of A-431 cells with antibodies *KS-B17.2*, *KS-1A3*, and *KS-2.1*. Negative staining of cells occurred with rabbit vimentin antibodies; *Bar*, 10 μm

knife. The ultrathin sections were examined in a Philips 410 T electron microscope at 80 kV.

Results

Polypeptide specificity of the monoclonal antibodies

Antibody KS-1A3. Western-blot analysis of A-431 cell cytoskeleton separated by two-dimensional gel electrophoresis revealed specific reactivity of this antibody with cytokeratin no. 13 (Fig. 1a, b). Further Western-blot analysis of cytokeratins from a variety of human tissues, together containing all 19 cytokeratins (including epidermis, vagina, cornea, exocervix, stomach, and cell lines A-431, MCF-7, and PLC) failed to disclose reactivity of this antibody with any other cytokeratin polypeptide. Immunohistochemical staining of cell line A-431 revealed positive staining (Fig. 2b). Immunohistochemical labelling of various epithelia with this antibody indicated that simple epithelial cells from a variety of tissues including colon (Fig. 3c), and liver, pancreas, and kidney tubules (data not shown) were negative. Pseudostratified epithelium of the trachea (Fig. 3i), the transitional epithelium of the urinary bladder (data not shown) and of the anus (Fig. 3l), squamous epithelium of the exocervix (Fig. 4a, see below), and tongue (data not shown) were extensively labelled. Myoepithelial cells of apocrine and eccrine sweat glands (Fig. 3f) were also positively labelled with this antibody. In epidermis, only the basal layer reacted strongly while the suprabasal layers were negative (data not shown). Interestingly epidermis and cornea were positively labelled using antibody *KS-1A3* by immunofluorescence, though no cross-reacting polypeptide could be detected in them by immunoblotting analysis (see below).

Antibody KS-B17.2. Using two-dimensional gel electrophoresis we determined that *KS-B17.2* antibody specifically recognizes human cytokeratin no. 18 (Fig. 1c, d). *KS-B17.2* reacted positively with A-431 cells (Fig. 2a) and with essentially all simple epithelia examined. These included colon (Fig. 3b) and sweat glands (Fig. 3e) as well as liver, pancreas, and kidney epithelium (data not shown). The luminal cells within the pseudostratified epithelium of the trachea were also positive while the basal cells of this epithelium were essentially negative (Fig. 3h). Antibody *KS-B17.2* positively stained acini of tracheal glands (data not shown) and sweat glands, but did not significantly react with myoepithelial cells. All stratified squamous epithelia tested, including exocervix (Fig. 4b), tongue, esophagus, and cornea were essentially negative (data not shown). Occasionally, weak staining of the basal layer was seen.

Antibody KS-2.1. The polypeptide specificity of antibody *KS-2.1* has not been directly defined here, since this antibody failed to react with electrophoretically separated cytokeratin polypeptides in a Western-blot analysis. Immunofluorescence staining of A-431 cells with *KS-2.1* and rabbit anti-vimentin revealed negative staining with anti-vimentin (Fig. 2c, d) while double staining of the cells with *KS-2.1* and rabbit anti-cytokeratin showed positive staining of the same filaments. By immunofluorescence, colon stained positively (Fig. 3a) and liver cells were negative except for small bile ducts, which were weakly positive (data not shown). Pancreatic acini and ducts, as well as kidney tubules, were also negative (data not shown). Tracheal pseudostratified epithelium and the transitional epithelium of the anal canal were both positive (Fig. 3g, j), while the squamous epithelium of the tongue was only faintly stained with this antibody. Myoepithelial cells of sweat glands, and also myoepithelial cells of tracheal acini, were intensely labelled (Fig. 3d).

Table 1. Reactivity of cytokeratin antibodies with endo- and exocervical cells.

	KS-1A3	*KS-2.1*	*KS-B17.2*
Endocervix			
Lumenal cells: Mucus secreting	−	−	+
Ciliated cells	−	+	+
Reserve cells	+	+*	−
Exocervix			
Basal layer cells	+	+	−
Suprabasal cells	+	−	−

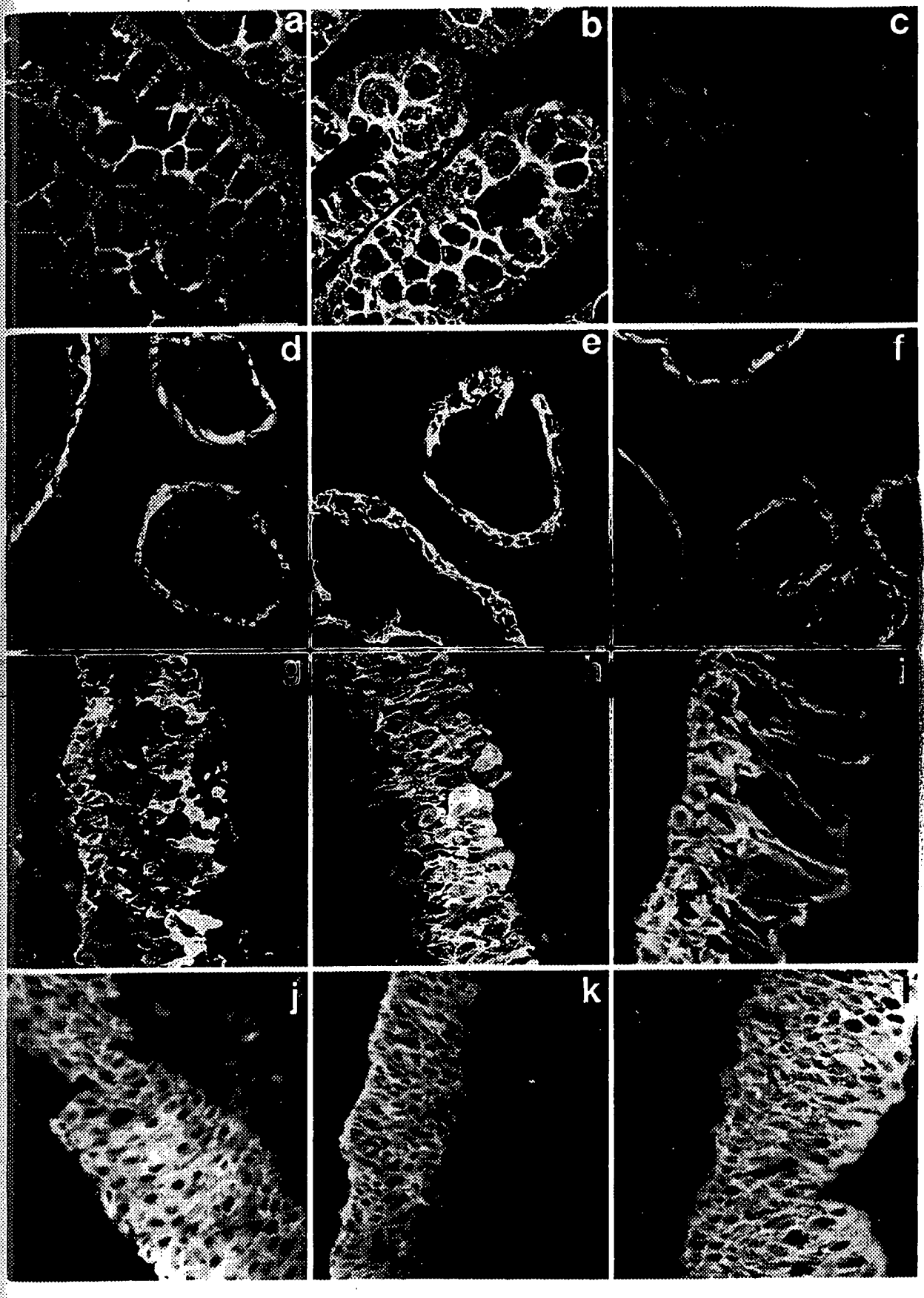
+, positive; −, negative
* Positive in six cases only

Cytokeratin expression in normal cervical epithelium

In frozen sections of various cervical regions, taken from 20 patients, labelling with the three cytokeratin-specific monoclonal antibodies described above revealed distinct labelling patterns, as summarized in Table 1. The normal exocervix showed positive staining throughout its layers using antibody *KS-1A3* (Fig. 5a). No labelling of the exocervix was obtained with antibody *KS-B17.2* (Fig. 4b), and *KS-2.1* decorated only the basal layer (Fig. 6a).

Cells facing the lumen of the normal endocervix, as well as those lining endocervical glands, were positively stained with antibody *KS-B17.2* (Fig. 4a, d) while no labelling was usually detected with either antibody *KS-2.1* (Fig. 6c, d) or *KS-1A3* (Fig. 5c). In six cases we obtained strong positive staining of isolated endocervical cells with antibody *KS-2.1* (Fig. 6e). Electron-microscopic examination indicated that these cells were of the ciliated type (Fig. 6f) [9]. The identification of ciliated cells was also assisted by the

Fig. 3a–l. Normal human tissues stained with monoclonal cytokeratin-specific antibodies. **a–c** Colon. **d–f** Sweat glands. **g–i** Tracheal pseudostratified epithelium. **j–l** transitional epithelium of anus. **a, d, g, j** Stained with antibody *KS-2.1*. **b, e, h, k** Stained with antibody *KS-B17.2*. **c, f, i, l** Stained with antibody *KS-1A3*. Note positive staining of the simple epithelium of colon with antibodies *KS-B17.2* and *KS-2.1*, while negative reaction is seen with antibody *KS-1A3*. Myoepithelial cells of sweat glands were positively stained by antibodies *KS-2.1* and *KS-1A3*, while antibody *KS-B17.2* stained only in luminal cells. Pseudostratified epithelial of the trachea was stained throughout with antibodies *KS-1A3* and *KS-2.1*, while antibody *KS-B17.2* did not stain the small cells in the base of the pseudostratified epithelium. Transitional epithelium of the bladder and of the anus was strongly stained by antibodies *KS-1A3* and *KS-B17.2* and somewhat less strongly with antibody *KS-2.1*. *Bars* represent 50 μm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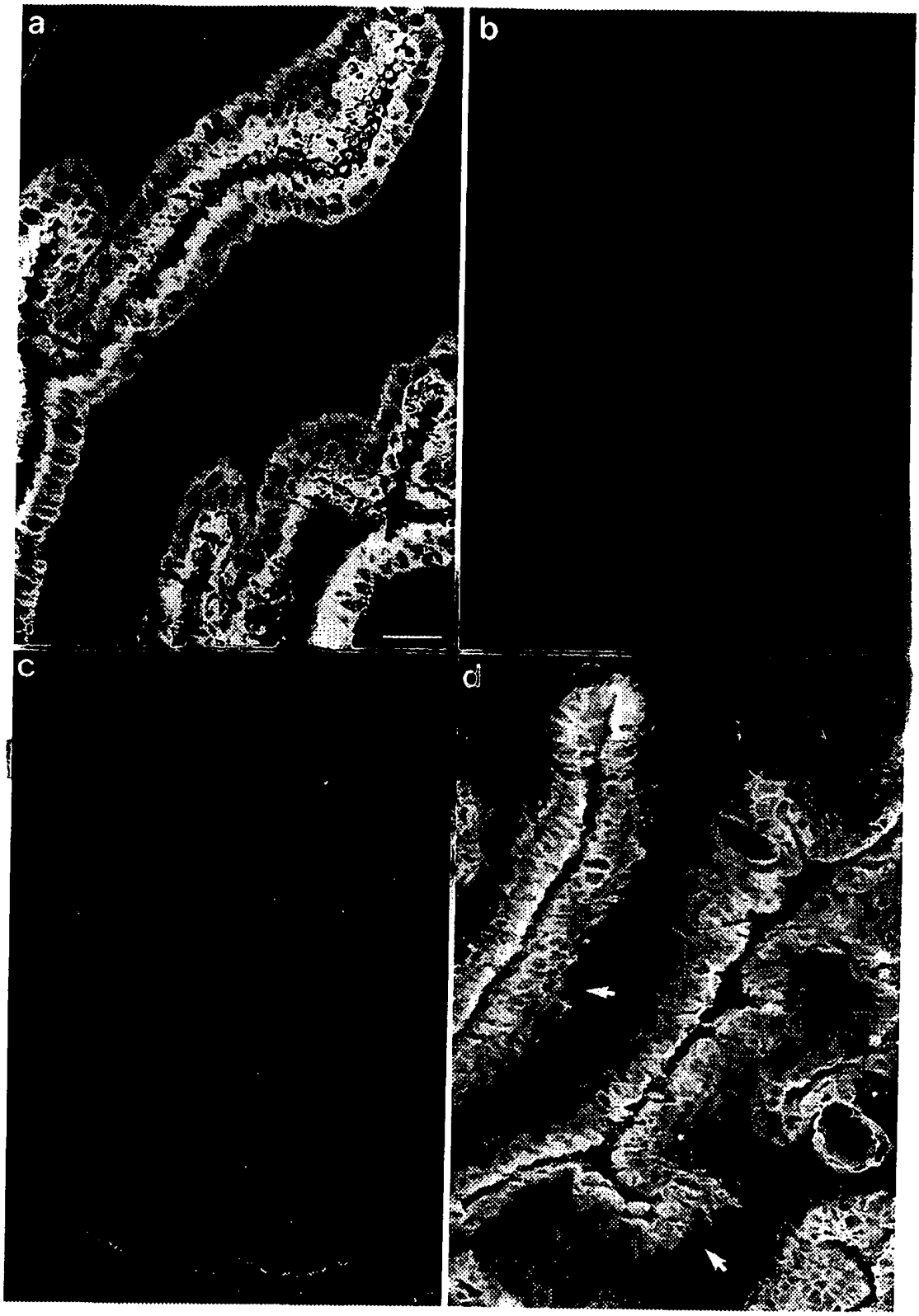

Fig. 4a–d. Immunofluorescence staining of various uterine cervical areas with monoclonal antibody *KS-B17.2*. **a** Endocervix without reserve cells. **b** Exocervix. **c** Cervical metaplasia. **d** Endocervix with reserve cells. Note negative staining of reserve cells and strong staining of columnar cells. Both metaplasia and exocervix are negative. Arrowheads in d point to reserve cells. *Bars* represent 50 μm

Fig. 5a–c
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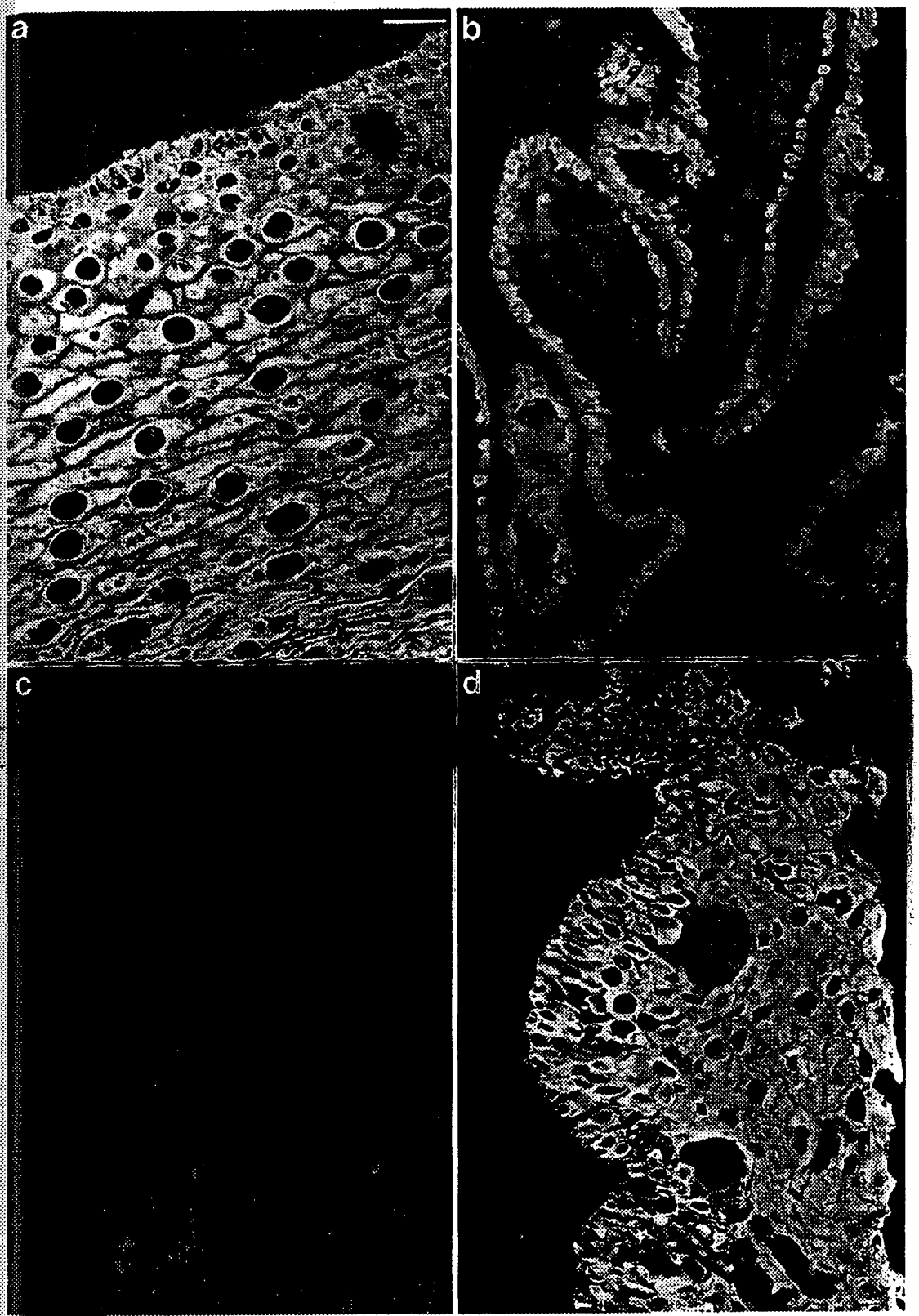

Fig. 5a–d. Immunofluorescence staining of various uterine cervical areas with monoclonal antibody *KS-1A3*. **a** Exocervix. **b** Endocervix with reserve cells. **c** Endocervix. **d** Cervical metaplasia. Note that the endocervical columnar cells are not stained while reserve cells are stained positively. Metaplasia is positively stained. *Bars* represent 50 μm

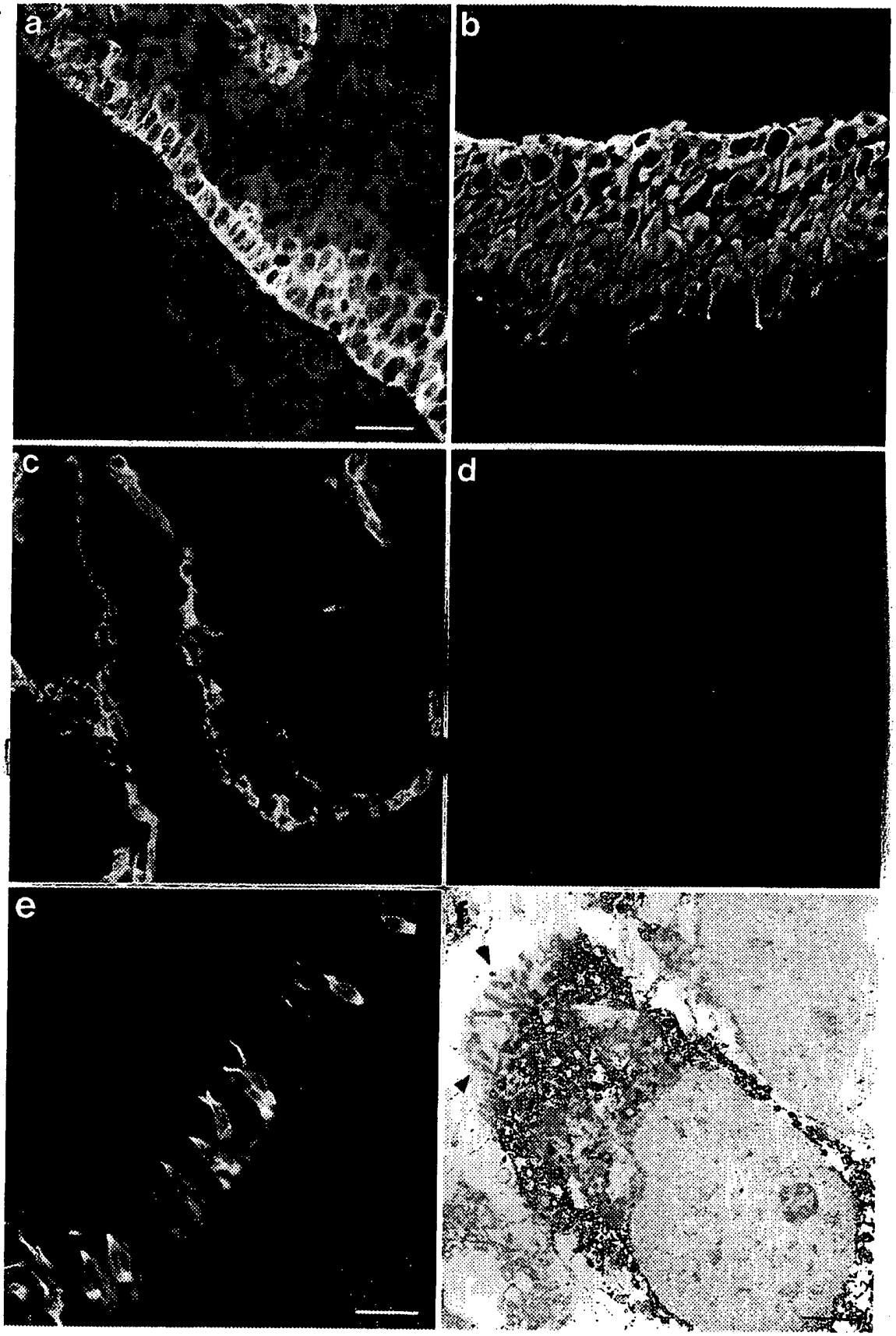

Fig. 6a–f. Immunofluorescence staining (**a–e**) and combined immunoperoxidase staining and electron microscopy (**f**), of various cervical areas with monoclonal antibody *KS-2.1*. **a** Exocervix. **b** Cervical metaplasia. **c, d** Endocervix with reserve cells. **e, f** Endocervix. Note different staining with this antibody in various areas of endocervix. In (**d**) note negative staining of reserve cells. In (**e**) note positive staining of ciliated cells. In (**f**) note specific staining of ciliated cells shown by electron-microscopic labelling of ciliated cells. Arrowheads in f point to cilia. *Bars* represent 50 μm in (**a–d**), 25 μm in (e) and 0.1 μm in (**f**)

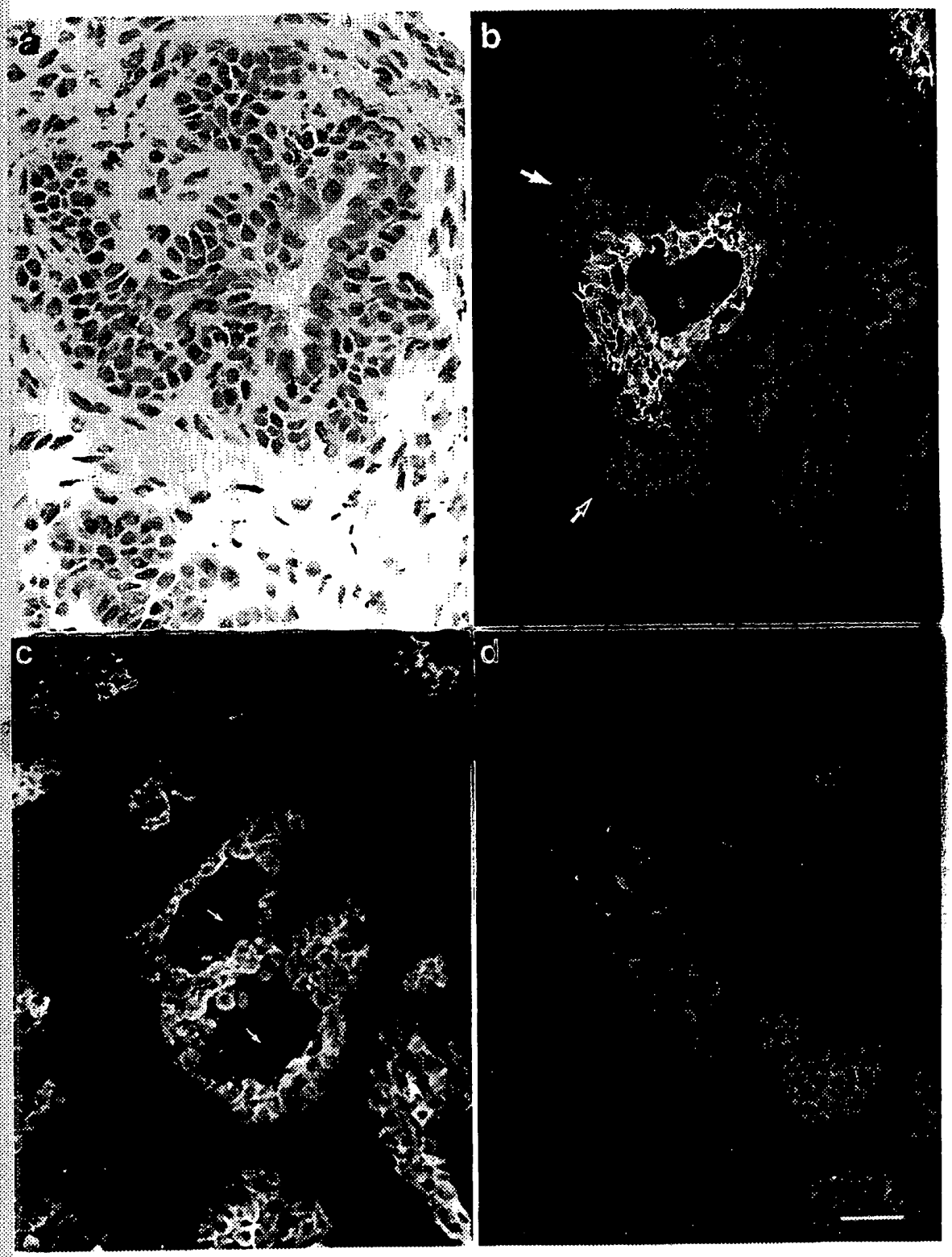

Fig. 7a–d. Hematoxylin-eosin-stained sections (**a**) and immunofluorescence microscopy (**b–d**) of endocervix with hyperplastic reserve cells. The antibodies used were: *KS-B17.2* (**b**), *KS-1A3* (**c**), *KS-2.1* (**d**). Note extensive staining of columnar cells with antibody *KS-B17.2*, while reserve cells are negative. Antibody *KS-1A3* shows staining of reserve cells and does not stain columnar cells. Arrowheads in b point to reserve cells; in c arrows point to columnar, unstained cells. *Bar* represents 50 μm

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use of monoclonal antibody directed against tubulin on adjacent sections (data not shown).

Reserve cells of endocervical glands found in 9 out of 20 cases examined were strongly stained with antibody *KS-1A3* (Fig. 5b). Labelling of reserve cells with antibody *KS-2.1* (Fig. 6c) was present in only six cases, suggesting that only a subpopulation of these cells contained the relevant epitope. Antibody *KS-B17.2* stained positively in the columnar cells, as indicated above, whereas the reserve cells were apparently negative (Fig. 4d).

Staining of hyperplastic reserve cells with the various cytokeratin antibodies revealed positive reaction with antibody *KS-1A3* (Fig. 7c). Both the columnar and the reserve cells in these regions stained faintly with antibody *KS-2.1* (Fig. 7d), while *KS-B17.2* stained columnar cells strongly. There was very weak or no staining at all of reserve cells with this antibody (Fig. 7b).

Cytokeratin expression in squamous metaplasia of the human cervix

Metaplastic lesions located near the transformation zone (exocervical – endocervical junction) or in other regions along the endocervix were strongly stained with antibody *KS-1A3* (Fig. 5d). There was no apparent labelling of metaplastic cells with antibody *KS-B17.2* (Fig. 4c), except for areas in which residual glandular elements were detected within the metaplastic areas (not shown). Cervical metaplasia was invariably positively stained with *KS-2.1* (Fig. 6b).

Discussion

In our previous study on the expression of cytokeratins in the human uterine cervix we have shown that all cell types in the normal endocervix were similarly labelled with the antibodies tested [21]. Metaplastic cells, on the other hand, presented a unique cytokeratin labelling pattern with the antibodies used in that study, distinct from that obtained with all other epithelial cells of the cervix. This finding suggested that metaplastic transformation involves the acquisition of a new route of differentiation distinct from that found in the normal cervix. The application of additional antibodies, as described in the present paper, compels us to partly revise this view. The use of the three new antibodies indicated that metaplastic cells express cytokeratin subsets similar to that of at least a subpopulation of reserve cells (see below) and enabled us to clearly distinguish between four types of cell within the normal endocervix. These included: (a) reserve cells that stained with antibody *KS-1A3* and were not labelled with antibody *KS-2.1*; (b) a subpopulation of reserve cells that was positively labelled with both these antibodies; (c) ciliated cells of the endocervical mucosa, which were the only luminal cells positively labelled with antibody *KS-2.1*; (d) columnar, nonciliated cells of the mucosa, which were positive only with antibody *KS-B17.2* and did not react with the other two antibodies tested here.

The presence of cytokeratin no. 13 in reserve cells, as described here, was surprising in view of our previous results, which showed that antibody *KS-8.12* (reactive with both polypeptide no. 13 and no. 16) did not stain these cells. We do not know the reason for the apparent discrepancy between the present and the previous results, yet we believe that selective epitope exposure may play a major role in determining the specific immunoreactivity of cytokeratins in the different cell types using different monoclonal antibodies. This phenomenon of masking and unmasking of specific cytokeratin epitopes has been described previously for other antibodies. It has been shown, for example, that the 50-kDa keratin polypeptide that is present throughout the various layers of the epidermis may be detected by antibody AE1 only in the basal layer of the epidermis. The specific epitope on this keratin was most probably masked in situ in the suprabasal cells [46]. In addition, it has been shown that the antigenic determinant recognized by antibody *KG 8.13* in PtK2 cells is masked in interphase cells and becomes available to this antibody only during mitosis or when the cytoskeleton is treated with various chaotropic agents [14]. Therefore, it appears that negative labelling with a specific cytokeratin antibody may not always indicate the absence of the respective polypeptide, but may sometimes involve an epitope-masking event. This phenomenon should be considered when immunohistochemical results are evaluated. It should be mentioned that a selective loss of immunoreactivity may not only occur in immunohistochemically examined samples, but may also be detected by immunoblotting analysis. Thus monoclonal antibody *KS-1A3*, used here, reacts on immunoblots with cytokeratin no. 13 only, although it reacts with tissue sections of cornea and epidermis that do not contain this polypeptide.

A comparison of our antibody *KS-1A3* with other antibodies reported as being directed against cytokeratin polypeptide no. 13 revealed differences in immunohistochemical staining. Thus, while *1C7* and *2D7* antibodies, which recognize cytokeratin no. 13, were negative in epidermis [31], our antibody stained basal layer of epidermis strongly, as did antibody *8.58* (directed against polypeptides no. 13 and no. 16) [6]. Negative staining of basal layer of squamous epithelium of anus and esophagus, was reported with *2D7* and *1C7*, while with *KS-1A3* this tissue was positive throughout all layers, including basal layer. Similar discrepancies were also seen in staining of cornea and urinary bladder. It is noteworthy that Caselitz et al. [5] recently reported a staining pattern similar to that of our antibody *KS-1A3*, in the uterine cervix, with the use of antibody *CKB1*. The polypeptide specificity of this latter antibody was defined as no. 14. Our antibody *KS-1A3* shows similar staining to antibody *KA1* [33], which stained basal layer of epidermis, all layers of exocervix, myoepithelial cells of sweat glands, tracheal glands, reserve cells of trachea, and endocervix and was negative with liver, kidney, colon, and thyroid. Differences between *KS-1A3* and *KA1* were seen only in surface epithelium of trachea, which was stained with *KS-1A3* and was negative with *KA1*. Reaction with *KA1*, as seen by immunoblot analysis of plantar foot epidermis, showed two bands that had molecular weights of 54 and 50 kDa [32]. Later, two additional bands from the same tissue were reported to react, with molecular weights of 58 and 56 kDa [33]. Nagel et al. [32] suggested that *KA1* recognizes a conformation-dependent epitope, on cytokeratin nos. 4, 5, and 6, that is expressed only in the presence of other unidentified components.

In view of our findings indicating that squamous metaplasia invariably stained with antibody *KS-2.1*, which also labelled a subpopulation of reserve cells, it can be proposed that the latter are either the progenitor cells of squamous metaplasia or represent a premetaplastic stage in reserve-cell development.

Another population of endocervical cells (beside the reserve cells) that apparently display a unique labelling pattern comprises the ciliated cells of the mucosa. These cells are the only luminal cells within the endocervix that are positively labelled with antibody *KS-2.1*. The nature of the positive cells was verified by complementary staining with anti-tubulin, enabling visualization of cilia, as well as by immunoelectron-microscopic examination of frozen sections. It should be mentioned that using an antibody to cytokeratin no. 4, Franke et al. [15] observed staining of individual columnar cells in several endocervical samples. They suggested that these individual cells may be the precursors of endocervical metaplasia. However, based on their morphological appearance, we would like to propose that these cells may be identical to those labelled with antibody *KS-2.1*, namely the ciliated endocervical cells. Furthermore, we do not consider these highly differentiated ciliated cells to be likely precursors of squamous metaplasia, which develops below the simple endocervical epithelium.

It should also be pointed out that the subpopulation of reserve cells labelled with antibody *KS-2.1* bears, in this respect, a similarity to the endocervical ciliated cell population. Whether this indicates that the subtype of reserve cells is capable of differentiation into ciliated cells can only be surmised.

As pointed out above, comparison of the immunofluorescent labelling of neighboring sections with antibodies *KS-1A3* and *KS-2.1* suggest that the reserve cells themselves may be heterogenous with respect to their cytokeratin composition or to the specific expression of a specific cytokeratin epitope. It was noted that the reserve cells were always intensely stained with antibody *KS-1A3* (Fig. 5b), while they were labelled with *KS-2.1* antibody (Fig. 6c) in some areas but not in others. Another notable observation in the present study was that both antibodies *KS-2.1* and *KS-1A3* recognize cytokeratins of myoepithelial cells in various glands. Our results with myoepithelia of salivary, mammary, and sweat glands showed a cytokeratin-polypeptide staining pattern similar to those reported by other groups [4, 7, 32, 38].

Our new immunohistochemical data based on reactivity with antibodies *KS-1A3* and *KS-2.1* favor the hypothesis that squamous metaplasia originates in reserve cells, and that reserve-cell hyperplasia represents an intermediate stage in this process [10]. Recently, Weikel et al. [45] published a study concerning cytokeratin expression of reserve cells. They used, among other antibodies, antibody *KA1*, which they described as an antibody reacting with cytokeratin filaments in stratified squamous epithelia, a reaction which was largely similar to that of our *KS-1A3*. On the basis of their findings, they suggested that reserve cells are derivatives of luminal cells rather than their precursors. Furthermore, in that study no labelling of cytokeratin polypeptide no. 13 in reserve cells was observed. This finding, which is in contrast to our observation, might be explained on the basis of epitope masking, or may have been due to small amounts of this cytokeratin in the tissue.

The present results suggest, therefore, that the metaplastic cells in the endocervix largely retain the cytokeratin polypeptides of the reserve cells from which they are derived, though the assembly of cytokeratins appears to undergo some changes, leading to altered exposure of epitopes and thus to distinct patterns of labelling with certain monoclonal antibodies.

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